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Influential Parameters on Biomethane Generation in Anaerobic Wastewater Treatment Plants

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1. Introduction

The nature of wastewater and its complexities are clear to all research scientists. The impact of industrial wastes on our environments is based on their characteristics and chemical composition as well as their degradability and treatment through biological processes. Application of biogas as a source of energy for heating and household usage has been promoted as an alternative solution to energy demands (Metcalf & Eddy, 2003).

In anaerobic fermentation process, generally four groups of microorganisms are sequentially active leading to the biodegradation of organic matters. In the process of anaerobic decomposition, organic compounds are hydrolyzed in the primary stage, as a result, long chain of aliphatic and aromatic organic compounds break down to small size volatile organic compounds and volatile fatty acids. Hydrolytic microorganisms are naturally capable of degrading biomaterials and organic compounds in wastewater. As a matter of fact, the hydrolysis takes place at the initial stage and the hydrolytic enzymes create a suitable environment for acid forming bacteria while intermediate metabolites are synthesized. In other word, large molecules; long chain natural polymers such as polysaccharides and proteins break down to their soluble and insoluble monomers. The monomers are then converted into fatty acids with small amount of hydrogen. The most frequently detected organic acids are acetic, propionic and butyric acids with small quantities of valeric acid produced. This stage is called acetogenesis (acid formation). The bacteria, producing acetic acid is called acetogenic bacteria and their optimal pH value is in the range of 5-6. Therefore, the main products of acetogens are a number of organic acids, hydrogen and carbon dioxide (Najafpour et al. 2008). In the acidification of organic wastes the pH of media drops to acidic condition. In the next stage of digestion, consortia of microbial populations utilize organic acids and hydrogen, methane and carbon dioxide are formed. In fact, anaerobic process decomposes all organic pollutants of wastewater to methane and carbon dioxide.

Anaerobic processes are normally operated at a pH near 7 which is in favor of methane formers, responsible for the final conversion of organic acids into methane. The final stage of anaerobic digestion is called methanogenesis. The product of acid forming phase, acetic acid mainly is converted into methane and carbon dioxide. The bacteria utilizing acetic acid, is called acetophilic bacteria. Alkalinity and hydrogen formers may seriously act on the bases of commensalisms which exhibit quite satisfactory coexistences with the acid formers. Energy is liberated through catabolic reactions while organic compounds are decomposed. Microorganisms grow on wastewater, using soluble organic compounds as sole source of energy for treatment and the useful end-product such as methane can be used as energy sources to generate electricity and heat. Moreover, it has been frequently reported that anaerobic treating of various wastewaters has achieved considerably high percent removal of chemical oxygen demand (COD)(>90%) (Nandy et al. 1998; Metcalf & Eddy 2003; Najafpour et al. 2005; Najafpour et al. 2006; Khademi et al. 2009; Zinatizadeh et al. 2009; Alrawi et al. 2010).

The purpose of the present chapter is to investigate the influential parameters on biomethane generation and efficient treatment of various wastewaters such as granulation, lipids in particular long chain fatty acids, organic loading rate, mixing, sludge recycling, temperature, alkalinity and pH, ammonia, and trace metal ions. Moreover, the applications of modern modeling techniques in providing support to the plant operation and the decision-making process are also discussed in detail.

2. Factors affecting biomethane generation

2.1 Granulation

The retention of a considerable amount of active biomass as happens in a number of reactor such as up-flow anaerobic blanket reactor (UASB)(Table 1), anaerobic baffled reactor (ABR) and upflow anaerobic sludge fixed film reactor (UASFF) is known as granulation. These anaerobic granules harbor several metabolic groups of microorganisms, including hydrolytic, fermentative, syntrophic, and methanogenic microorganisms, involved in the anaerobic degradation of complex organic compounds (Satoh et al., 2007). Granules characteristically have a spherical form with a diameter from 0.14 to 5 mm (Schmidt and Ahring, 1996). Granules have been reported to be in different colors i.e. black, gray and brown reflecting the different stages in their life cycle (Liu, 2003). The spatial distributions of important phylogenetic groups in the granules have been determined by fluorescent *in situ* hybridization (*FISH*) (Santegoeds et al., 1999; Ariesyady et al., 2007; Satoh et al., 2007). In situ hybridization results showed that the outer layer of the granules was dominated by *Bacteria* whereas the inner layer was dominated by *Archaea* affiliated with *Methanosaeta* (>70%) (Santegoeds et al., 1999; Sekiguchi et al., 1999). However, the center of the granules was composed of dead or resting cells, or both, which were used as a support for active archaeal and bacterial cells near the surface (Yuko Saiki et al., 2002; Díaz et al., 2006).

For start-up of reactors in which granules are present such as UASB, the development of stable granules is the key factor for successful operation (Sekiguchi et al., 2001). Several factors are known to have prominent effects on the granulation process including hydrophobicity of constituents in sludge such as surface of cells (Daffonchio et al., 1995; Schmidt and Ahring, 1996), the presence of extracellular polymers (ECP) (Schmidt and Ahring, 1996; Veiga et al., 1997), and the composition of different layers and microorganisms (Wiegant and de Man, 1986; Schmidt and Ahring, 1996; Sekiguchi et al., 2001; Yuko Saiki et al., 2002). Among these, the microbial constituents are thought to be the decisive factor in many cases.

As CO_2 and CH_4, the main biogases produced during biomethanation (Tabatabaei et al, 2010) are poorly water-soluble, granular sludge with a very hydrophobic surface obviously tend to float (Daffonchio et al., 1995). The latter could adversely affect biomass retention in self-immobilized cell systems, such as UASB reactors (Daffonchio et al., 1995). Therefore, the formation of consortia with more hydrophilic cells such as *Chloroflexi* in the outer layer is of great importance. In addition, granules containing methanogens deep within them as well as those with a *Bacteria* layer on the surface tend to float, therefore, well-settled granules are considered to have methanogens that develop near the granule surface so that the gases generated during methane fermentation are readily released (Yuko Saiki et al., 2002).

The extracellular polymers in the granular sludge vary between 0.6 and 20% of the volatile suspended solids (VSS) and consists mainly of protein and polysaccharides (Schmidt and Ahring, 1996). These extracellular polymers are important for the structure and maintenance of granules (Veiga et al., 1997). *Methanosarcina* spp. and more importantly *Methanosaeta* spp. have been identified as essential aceticlastic methanogens for the initial granulation and development of granular sludge under mesophilic conditions (Schmidt & Ahring, 1996). They are considered of significant importance for making cores of sludge granules by constructing structures similar to a spider's web (Sekiguchi et al., 2001).

In contrast, in granules under thermophilic conditions, the long-filament type of *Methanosaeta* cells are replaced by dispersed type of *Methanosaeta* cells (Uemura & Harada, 1995; Syutsubo et al., 1997). Therefore, in thermophilic granules, the settleability of the granules is linked with the predomination of another group of very thin filamentous microorganisms instead of *Methanosaeta* (Sekiguchi et al., 2001). This type of microbe which is affiliated with a Clone Cluster, the Green Non-Sulfur Bacteria, Subdivision I, entirely covers the thermophilic granules, forming a web-like outside layer on granules (Uemura & Harada, 1993; Uemura & Harada, 1995; Sekiguchi et al., 1999; Sekiguchi et al., 2001). A combination of volatile fatty-acid mixtures and an addition of sucrose or glucose to the influent wastewater seemed to be essential for the optimum growth of these microbes and consequently the formation of granular sludge with good settleability (van Lier et al., 1994; van Lier et al., 1996).

Several studies have shown that the granulation process in different reactors such as anaerobic sequencing batch reactors (ASBRs) (Wirtz & Dague, 1996; El-Mamouni et al. 1997), ABR (Uyanik et al., 2002) and UASB reactors (Tiwari et al., 2004; Tiwari et al., 2005) can be influenced by conditioning the sludge with polymers. A study conducted by Uyanik et al. showed that a polymer-amended ABR reactor contained sludge that had a greater density of anaerobic bacteria and larger and denser granules than the control reactor, indicating that polymer addition possibly contributed to the retention of active biomass and resisted washout within the ABR (Uyanik et al., 2002). Moreover, it was found that the time required to form granules in a polymer-amended ASBR by using powdered activated carbon was reduced by approximately 75% compared to a control reactor (Wirtz & Dague, 1996). In fact, freely moving polymeric chains may form a bridge between cells, and this would facilitate the formation of initial microbial nuclei, which is the first step towards granulation (Liu et al., 2003). This is especially essential for UASB reactors which in them the long start-up period required for the development of anaerobic granules seriously limits the application of this technology. Therefore, it appears that polymers (synthetic and natural) can assist anaerobic bacteria to aggregate together and then form granules faster.

Abbreviation	Definition	Abbreviation	Definition
ABR	anaerobic baffled reactor	MAPE	mean absolute percentage errors
AFB	anaerobic fluidized bed	MLP	multilayer perceptrons
AF	anaerobic filters	MOO	multi-objective optimization
ANFIS	adaptive network based fuzzy system	MSE	mean squared error
ANN	artificial neural networks	OLR	organic loading rate
ASBRs	anaerobic sequencing batch reactors	ORP	oxidation-reduction potential
BOD	biological oxygen demand	PCA	principal component analysis
COD	chemical oxygen demand	POME	palm oil mill effluent
CSTR	completely stirred tank reactor	SBR	sequencing batch reactor
EGSB	expanded granular sludge blanket	STP	standard temperature and pressure
EPA	Environmental Protection Agency	TAN	total ammonia nitrogen
FISH	fluorescent in situ hybridization	TVFA	Total volatile fatty acids
Ga	genetic algorithm	UASB	up-flow anaerobic blanket reactor
GM-ANN	Grey Model ANN	UASFF	upflow anaerobic sludge fixed film reactor
HLR	hydraulic loading rate	VFA	Volatile Fatty Acids
HRT	hydraulic retention time	VSS	volatile suspended solids
KSOFM	Kohonen self-organizing feature maps	WTP	wastewater treatment plant
LCFA	long chain fatty acids		

Table 1. List of the abbreviations and definitions

2.2 Lipids in particular long-chain fatty acids (LCFAs)

Lipids, largely in the form of neutral fats, are commonly present in domestic sewage and industrial effluents such as food-processing wastewater (for instance dairy wastewater), wool-scouring wastewater, slaughterhouse wastewater and edible oil-processing effluents (Sousa et al., 2009). Lipid content and composition of industrial wastewaters is considerably variable. Lipid concentration of 0.2-1.3 g/l was measured in wastewaters from a sunflower oil mill (Saatci et al., 2003). Relatively higher levels of lipids were detected in wastewaters from a dairy industry (1.5–4.6 g/L) (Mendes et al., 2006). Becker et al. and Beccari et al. reported the presence of the minimum of 10 g/l and 16 g/l of lipids in olive oil-processing and wool-scouring effluents, respectively (Becker et al., 1999; Beccari et al., 2002). Furthermore, wastewaters significantly differ in their LCFA composition but in general palmitic and oleic acids are the most abundant saturated and unsaturated LCFA, respectively (Sousa et al., 2009).

Lipids are in general, glycerol bonded to LCFA, alcohols, and other groups by an ester or ether linkage. Triacylglycerides (neutral fats), are the most abundant family of lipids and are hydrolyzed by extracellular lipases to glycerol and LCFA. Glycerol is further degraded via acidogenesis while LCFA are broken down to acetate, H_2 and CO_2 through the β-oxidation process (syntrophic acetogenesis) (Stryer, 1995).

The application of anaerobic digestion of fat-rich wastewaters was hindered by problems related to LCFA adsorption to the biomass, with consequent sludge flotation and washout (Rinzema et al., 1993; Hwu et al., 1998a; Hwu et al., 1998b) and inhibitory or toxic effects of LCFA to different groups of microorganisms such as acetoclastic methanogens (Koster & Cramer, 1987; Rinzema et al., 1994; Angelidaki & Ahring, 1995; Lalman & Bagley, 2001) and to a much less extent to hydrogenotrophic methanogens (Hanaki et al., 1981; Lalman & Bagley, 2001). Besides the potential metabolic inhibition, LCFA accumulation onto the sludge can create a physical barrier or damage the cell wall, with consequent limitations in the transport of substrates and products (Pereira et al., 2005). Hence, treatment of fat-rich effluents in order to reduce the fat concentration before the anaerobic digestion was advised (Perle et al., 1995). However, it consequently leads to loss of their energetic potential as theoretically at standard temperature and pressure (STP), 1 g of oleate (unsaturated LCFA, C18:1) results in approximately 3 times higher methane production than 1 g of glucose (Cavaleiro et al., 2008) marking them attractive sources for methane production (Kim et al., 2004; Pereira et al., 2004). This potential is however limited due to the above mentioned operational problems.

Several technologies have been proposed for the anaerobic treatment of oily effluents, namely UASB reactors (Kim et al., 2004; Jeganathan et al., 2006), expanded granular sludge bed (EGSB) reactors (Hwu et al., 1998; Pereira et al., 2002a), and anaerobic filters (AF) (Pereira et al., 2002a). However, as explained earlier, due to fat-related operational problems, that efficient treatment of LCFA-rich wastewater can only be accomplished, if a correct equilibrium between accumulation and degradation is assured (Pereira et al., 2004; Cavaleiro et al., 2008). Different approaches has been practiced to achieve this equilibrium such as cycles of continuous LCFA feeding, followed by batch degradation of the accumulated substrate (Pereira et al., 2004; Pereira et al., 2005) and continuous operation after a step feeding start-up (Cavaleiro et al., 2006). Nielsen and Ahring also showed that the addition of oleate pulses to thermophilic reactors treating mixtures of cattle and pig manure had a promising effect on the overall process (Nielsen & Ahring, 2006). A study by Pereira et al. confirmed LCFA adsorption during continuous oleate feeding in anaerobic bioreactors (Pereira et al., 2004) which is a necessary state for LCFA degradation (Hwu et al., 1998b). They proposed a value of about 1000 mg COD-LCFA/g biomass for the optimal specific adsorbed-LCFA content that led to the maximal degradation rate. Long-term operation at higher LCFA-loading rates resulted in LCFA accumulation in bioreactors, with consequent limitations in the transport of substrate to the biomass and decreased removal efficiency (Pereira et al., 2004).

In addition, the organic suspended solids such as lipids and proteins present in seafood processing wastewaters are considered as a serious drawback for the use of high rate anaerobic reactors, such as UASB or AF, in which case the implementation of a previous hydrolysis–acidification step would be required (Guerrero et al., 1999). Therefore for this type of effluents, two-phase systems are recommended, since these compounds could be degraded to Volatile Fatty Acids (VFA) in the first reactor by hydrolytic and acidogenic bacteria and finally converted into methane in the second reactor (Guerrero et al., 1999). Tagawa et al. encountered severe operational problems and unsatisfactory COD removal efficiency (60 to 70%) during the anaerobic treatment of a food processing wastewater containing high strength of lipid and protein in a thermophilic multi-staged UASB reactor (Tagawa et al., 2002). They reported the formation of a severe scum and insolubilized substance within the UASB sludge bed due to the presence of high strength of lipid and protein along with high cations concentration including Mg and Ca in the raw wastewater. This resulted in hindering the contact efficiency between substrate and sludge. Moreover, the replacement of active microbial granules in the sludge bed with the insolubilized protein and lipid led to reduced methanogenic activity (Tagawa et al., 2002). Enzymatic pretreatment (lipases) was reported as a very promising alternative for treating wastewaters having high-fat contents (Cammarota & Freire, 2006; Mendes et al., 2006; Leal et al., 2006). Mendes et al. reported higher biogas formation due to lipids liquefaction and bioavailability for anaerobic microorganisms when a dairy wastewater (lipids, 1.5 to 4.7 g/l; free fatty acids, 5 to 69 mg/L) was pretreated with a low-cost lipase for 12 h (Mendes et al., 2006). In addition, high COD and color removal was achieved.

Cammarota et al. obtained promising results when evaluated the efficacy of an enzymatic hydrolysis stage in dairy industry wastewater prior to the anaerobic biological treatment using *Penicillium restrictum* lipases (Cammarota et al., 2001). The authors obtained COD and lipid removal efficiencies of 91% and 82% when the reactor was operated with low loads of lipids (203 mg lipid/L). However, the removal efficiencies diminished significantly when the reactor received wastewater containing high lipid levels (868 mg/L), with COD and lipids removal efficiencies climbing down 50% and 40% ,respectively, due to the toxic effects of LCFAs on the anaerobic consortia. When wastewater pre-treated with 0.1% (w/v) of fermented babassu cake containing *P. restrictum* lipases was fed, the average COD and lipid removal efficiencies rose to their initial values of 92% and 89%, respectively, within approximately 15 days (Cammarota et al., 2001). These findings support the idea of applying hydrolytic enzymes as coadjutants in the anaerobic treatment of fat-rich wastewaters (Cammarota et al., 2001; Leal et al., 2002). Taken all together, pretreatment of lipid- and protein-rich food industrial wastewaters is a perquisite to their successful anaerobic treatment.

Concerning the microbiological aspects of fat-rich wastewaters, about 14 species have been described with the ability to grow on fatty acids in syntrophy with methanogens, all belonging to the families *Syntrophomonadaceae* and *Syntrophaceae* in the subclass of the *Deltaproteobacteria* (Jackson et al., 1999; Sousa et al., 2009). Sousa et al. applied molecular approaches to investigate the microbial diversity of anaerobic sludge after extended contact with long LCFA and found the predominance of the members of the phylum *Firmicutes* (87%), among which members of the *Clostridiaceae* and *Syntrophomonadaceae* families represented 69% (Sousa et al., 2007). Several studies investigating the diversity of methanogenic archaea in up-flow bioreactors fed with oleate or palmitate found a predominance of hydrogenotrophic methanogens belonging to the genera *Methanobacterium* (Pereira et al., 2002b; Sousa et al., 2007). Similarly, in a study conducted by Shigematsu et al. on a chemostats fed with a mixture of oleate (C18:1) and palmitate (C16:0), hydrogenotrophic methanogens (genus *Methanospirillum*) were found predominant (Shigematsu et al., 2007). Acetoclastic archaea mainly *Methanosaeta* and *Methanosarcina* species (Pereira et al., 2002b; Shigematsu et al., 2007; Sousa et al., 2007; Tabatabaei et al., 2009) have an important role in the overall efficient conversion of LCFA to methane, because methane derived from acetate accounts for about 70% of the total theoretical methane potential of LCFA (Sousa et al., 2009). Pereira et al. explained the decrease of methanogenic activity after cell contact with LCFA temporarily mainly because of mass transfer limitations (Pereira et al., 2005). However, in different study, Pereira et al. (2004) reported increased specific methanogenic activity after LCFA adsorption to biomass followed by efficient conversion of biomass to methane (Pereira et al., 2004). Therefore, it could be concluded that a possible acclimation of methanogens takes place after contact with LCFA and consequently the adverse effects of LCFA on anaerobic sludge functionality are not irreversible and that anaerobic sludge, under the appropriate conditions reaching the mentioned equilibrium is able to efficiently mineralize them (Pereira et al., 2003). Sousa et al. reported the possibility of the domination of sulfate-reducing hydrogen and acetate consumers (*Desulfovibrio*-, *Desulfomicrobium*- and *Desulforhabdus*-related species) during the anaerobic treatment of sulphate-LCFA-rich wastewaters (Sousa et al., 2009).

2.3 Organic loading rate (OLR)

The OLR variation can be derived from either variation in influent COD or variation in flow rate with constant COD. An increase in OLR beyond the optimum level are followed by a decrease in the main process parameters such as COD removal, specific methane production. In addition, high amount of suspended solids "known as biomass wash-out" are observed in the effluent, indicating that the reactor suffered a process imbalance and that biomass accumulated in the reactor (Converti et al., 1993; Rizzi et al., 2006; Fezzani & BenCheikh, 2007; Rincón et al., 2008). This could be ascribed to an increase in the concentrations of the VFAs with a consequent decrease in pH (Tiwari et al., 2006) or to escalated levels of inhibitory or toxic compounds such as phenols, LCFAs, lignin and etc. Therefore, there is a maximal operational value for this parameter. For instance, Rizzi et al. reported a decrease in COD removal and specific methane production when OLR was increased from 10 to 15 g COD/l/day. With the OLR increase to 20 g COD/l/day the biomass excess started to wash out, followed by deterioration of the reactor performance (Rizzi et al., 2006). In a different study, stable reactor performance was observed when the OLR increased from 1.5 to 9.2 g COD/l/day with the maximum methane production rate achieved for an OLR of 9.2 g COD/l/day. However, a significant decrease in the pH value (from 7.5 to 5.3) was observed when OLR was further raised to 11.0 g COD/l/day. In addition, the increase in the effluent CODs with increased OLR was paralleled to a sharp increase in the effluent total volatile fatty acids (TVFA, g acetic acid/L) by about 400% (Rincón et al., 2008). This indicates that, at higher OLR, the effluent total COD and mainly soluble COD is largely composed of the unused volatile acids produced in the reactor due to the inhibition of methanogenesis.

Methanobacteriaceae and *Methanosaeta* were found the main methanogens in a laboratory-scale up-flow anaerobic digester treating olive mill wastewater (Rizzi et al., 2006). However, the authors also reported an interesting population shift by OLR variation. At lower OLR i.e.

6 g chemical oxygen demand (COD/l/day, hydrogenotrophic *Methanobacterium* predominated in the reactor but the number of cells/g sludge showed a 1000 fold decrease from 10^{11} to 10^8 when the OLR was increased to 10 g COD/l/day. In contrast, phylotypes belonging to the acetoclastic *Methanosaeta* were not affected by OLR variation and at 10 g COD/l/day, dominated in the biofilm (10^9 cells/g sludge) (Rizzi et al., 2006). Olive oil wastewater is characterized by high levels of inhibitory/toxic compounds such as tannins, and lipids. As a result, increased OLR leads to higher concentration of these substances and a consequent inhibition of methanogenic cells. However, acetoclastic *Methanosaeta* due to its high affinity for acetate is capable of occupying the deepest and thus more protected niches in the granule or biofilm with low concentrations of substrate (acetate) (Gonzales-Gil et al., 2001). Phylotypes belonging to the genus *Methanosaeta* were also dominant independent of different OLRs in other anaerobic digesters (Rincón et al., 2006; Rincón et al., 2008). So with the above findings, we may suggest that these acetoclastic methanogens i.e. *Methanosaeta* be the least susceptible methanogens to the elevated concentrations of toxic compounds when bioreactors are operated at high OLRs. In a different study, Kalyuzhnyi et al. investigated the microbial ecology of granules in UASB-reactor fed by synthetic wastewater under various OLRs. The authors showed that the predominant microbial biomass was *Methanosaeta*. However, increasing the OLR led to a substantial increase of *Methanosarcina* in the granules (Kalyuzhnyi et al., 1996). The increase of *Methanosarcina* in the studied synthetic wastewater (toxin-free) due to increasing OLR is explained by the low affinity of these methanogens for acetate in comparison with *Methanosaeta.* Hence, by increasing OLR and consequent VFAs concentration, *Methanosarcina* is favored. These findings would have been more interesting if they had included a group of inhibitory/toxic compound/s in the synthetic medium.

As reviewed earlier, under mesophlic conditions *Methanosaeta* plays a significant role in making cores of sludge granules (Schmidt & Ahring, 1996; Sekiguchi et al., 2001) and thus their ratio seems to control the speed of granulation (Rincón et al., 2006). Higher OLRs result in consequent higher concentrations of substrate i.e. acetate in the reactor. Morvai et al. attempted to investigate the influence of organic load ranging from 0.5 to 3.0g/1 on granular sludge development in an acetate-fed system (Morvai et al., 1990). They argued that in the range of feed acetate levels examined, higher concentrations of feed (acetate) caused faster granulation of the sludge bed and, presumably, of the microbial population, and resulted in better sludge structure and improved sludge settleability (Morvai et al., 1990). Low OLR has been reported to cause acute mass transfer limitation leading to disintegration of the larger granules (Ahn et al., 2002). The disintegration begins at the core of the granules due to substrate limitation with a consequent loss of granule's strength and stability (Kosaric et al., 1990). However, this was not in agreement with the studies conducted by Teer et al. and Tiwari et al. which low OLR (<1.5 kg COD/m^3/d) did not lead to disintegration of the granules in UASB reactors (Teer et al., 2000; Tiwari et al., 2005). This could be ascribed to the different experimental settings and wastewaters used in these studies. Teer et al. attempted to treat a high iron bearing wastewater in a UASB reactor. Evidence shows that the presence of divalent and trivalent cations ions, such as Fe^{2+}, and Fe^{3+}, helps bind negatively charged cells together to form microbial nuclei that promote further granulation (Teo et al., 2000). On the other hand, Tiwari et al. tried to enhance the granulation process by using natural ionic polymer additives. These may thus reduced the effect of low OLR (i.e. substrate limitation) on the granules and delayed the disintegration (Tiwari et al., 2004).

Converti et al. reported that COD removal rate, the COD specific removal rate (r_s) and methane production rate were not suppressed by increasing OLR when treating wine wastewater and sewage mixture (Converti et al., 1990). That indicated that no inhibition factor related to the organic content of the effluent was present in both wine wastewater and sewage mixture studied. This was further supported by the cell mass concentration varied very little with increasing the OLR. However, as completely noticed by the authors, even at the absence of inhibitory/toxic compounds, in the initial part, the removal rate increased with the OLR, following a first order kinetic. In the second part, instead, the removal rate, tended to a constant maximum value, following a zero order kinetic. Afterwards, the removal efficiencies as well as the methane production yield gradually decreased with increasing influent COD due to increasing the OLR, which evidently showed a substrate inhibition occurrence (Converti et al., 1990). This supports the idea that even at the absence of the inhibitory/toxic compounds in the wastewater, increasing influent COD by the means of increasing OLR could lead to substrate inhibition and consequent reduced removal efficiencies. Converti et al also described the observed dependence of the removal rate on the OLR by an empirical equation similar to Monod's model to compare the degradability of different effluents (see Equation 1)(Converti et al., 1990):

$$r_s = r_{s(max)} OLR / (k + OLR) \tag{1}$$

where $r_{s(max)}$ [kg COD/(kg_{vss} d)] is the maximum value of r_s, and k a constant which physically is expressed in units of OLR and an increase of k indicates increased treatment ability of the studied effluent.

Taken all into consideration, the desired OLR is the function of the favorable effect of OLR on stimulating the growth of methanogens in the bioreactor by providing them with higher substrate concentrations, its reverse effect on elevating the concentration of inhibitory/toxic compounds and the buffering capacity of methanogenic community. In the other words, the maximal operational value of OLR (OLR_{max}) is translated into the highest methane production (indicating the highest conversion efficiency of the system) that the buffering capacity of methanogenic community is still capable of compensating for elevated concentrations of inhibitory/toxic compounds.

2.4 Mixing

There is only a limited number of studies found specifically focused on the effects of mixing on the treatment efficiency and biogas production using various types of organic wastes including cow manure, palm oil mill effluent (POME), animal waste, municipal solid waste, primary sludge and fruit and vegetable wastes (Stafford, 1982; Stroot et al., 2001; Karim et al., 2005a; Karim et al., 2005b; Kaparaju et al., 2007; Sulaiman et al., 2009). Adequate mixing is very important in order to achieve successful anaerobic treatment of organic rich wastewater. In another word, it enhances the anaerobic process rate by preventing stratification of substrate, preventing the formation of surface crust, ensuring the remaining of solid particles in suspension, transferring heat throughout the digester, reducing particle size during the digestion process and releasing the biogas from the digester content (Kaparaju et al., 2008; Sulaiman et al., 2009).

Prior to 1950s, anaerobic digesters treating sewage sludge were not equipped with mechanical mixing and thus caused the formation of scum layer at the surface (Fannin, 1987). To overcome this problem, mixing was employed to disrupt scum formation and enhance contact between microorganisms and substrates. It has been reported that the acetate-forming bacteria and methane-forming bacteria are required to be in close contact to achieve continuous degradation of organic materials (Gerardi, 2003). In addition to the mentioned advantages, mixing also helps to eliminate thermal stratification inside the digesters, maintain digester sludge chemical and physical uniformity, rapid dispersion of metabolic products and toxic materials and prevent deposition of grit (Gerardi, 2003).
In modern anaerobic digesters, mixing could be achieved in various ways such as gas injection, mechanical stirring and mechanical pumping as presented in Table 2 (Tchobanoglous & Burton, 1991). In more advanced applications where higher temperature is needed, digester is also equipped with a heater used to heat up the digester content or feed as illustrated in Figure 1 (Gerardi, 2003). Generally for large scale applications, agitator or mixer system is commonly used to mix substrate homogenously inside the bioreactor and to provide a good contact between microorganisms and the substrate. All previously published articles on the effects of mixing on the stability and methane production reported similar observations which led to a consistent conclusion that vigorous mixing that is turbulent flow in nature is unsuitable for microorganisms growth and consequently results in an unsatisfactory methane production (Stafford, 1982; Stroot et al., 2001; Karim et al., 2005a; Karim et al., 2005b; Kaparaju et al., 2007; Sulaiman et al., 2009). This is basically due to the effect of high shear force on separating the hydrolytic bacteria from their substrate (Stafford, 1982). This is supported by another study on the effect of shear force on both aerobic and anaerobic sludge (Sheng et al., 2008). They found out that the anaerobic sludge or flocs (with shear sensitivity of 0.088) was less stable compared to the aerobic sludge flocs (shear sensitivity of 0.032). Through further investigation, Sheng et al. also discovered that the adhesion enthalpy based on the shear experiment (ΔH_G) for anaerobic flocs was larger (-919 l/s) than that of the aerobic flocs (-1600 l/s). This reflects that the deflocculating process is less energy demanding for anaerobic flocs or in other word, the flocs in the anaerobic sludge is easier to be broken than the aerobic flocs.

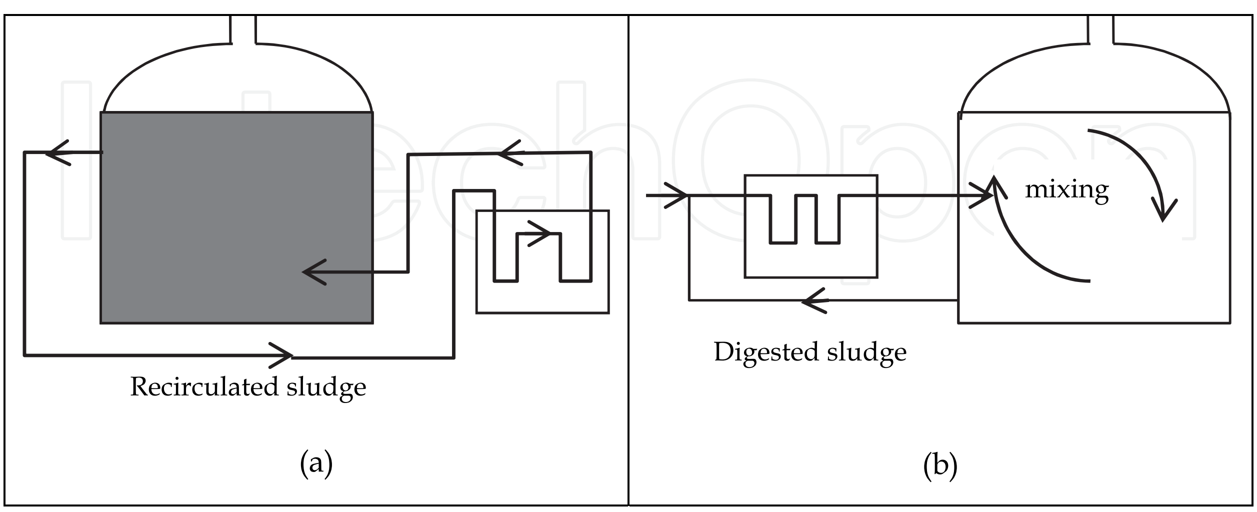


Fig. 1. The mixing methods that incorporate heating with limited mixing achieved through recycling sludge through a heat exchanger (a) and simultaneous mixing and heating of the digested sludge inside the bioreactor through sludge recycling system. (Gerardi, 2003).

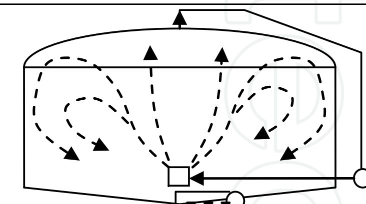
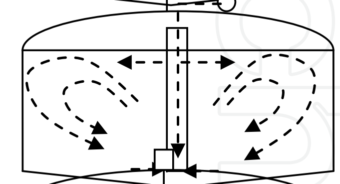
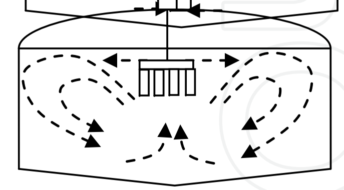
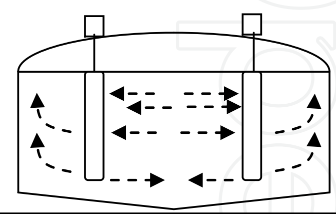
No	Type of Devices	Description	Type of digesters the device suits	Schematic Figure	References
1	Unconfined gas injection system	Gas is collected at the top, compressed, recycled and discharged at the bottom of the digester. The rising gas inside the digester will mix the content and the sludge.	Suitable for digesters with fixed, floating or gas holder covers such as continuous stirred tank reactor (CSTR).		Tchobanoglous and Burton, (1991)
2	Confined gas injection system	Gas in collected at the top, compressed and returned into through confined tubes.	Suitable for fixed, floating or gas holder covers such as continuous stirred tank reactor (CSTR).		Tchobanoglous and Burton, (1991)
3	Mechanical stirring system	Mixer is used to displace the sludge and mix the digester contents. The mixer usually has one cover-mounted mixer at the top of the digester.	Suitable for fixed or floating covers such as continuous stirred tank reactor (CSTR), sequencing batch reactor (SBR).		Tchobanoglous and Burton, (1991); Song et al (2004). Fannin and Biljetina (1987)
4	Mechanical pumping system	Turbine is installed which usually has one cover-mounted motor at the top and the turbine impeller is located at different sludge depths. Mixing pump may also be used for effluent recycling and mixing.	Suitable for fixed or floating covers such as continuous stirred tank reactor (with semi-continuous mixing system)		Sulaiman et al., (2009); Visser et al., (1993);

Table 2. Various mixing devices used in high rate anaerobic digesters systems

In addition to high turbulent mixing, continuous mixing could also reduce the performance of the biogas production as reported by Stroot et al. (2001) and Sulaiman et al. (2009). The former studied mixing organic fraction of municipal solid waste co-digested with primary sludge and waste activated sludge and found the continuous and vigorous mixing inhibitory when the system was operated at a high organic loading rate due to the disruption of syntrophic relationships and spatial juxtaposition of the microorganisms responsible for organic degradation and methane production (McMahon et al., 2001; Stroot et al., 2001). Sulaiman et al. investigated anaerobic treatment of POME for biogas production using a pilot scale bioreactor under constant mixing with turbulent flow regime condition and discovered that the methanogenesis process was totally inhibited just after 13 days of operation. The biogas was not produced at all and the VFA content was recorded higher than its inhibitory limit (i.e 1000 mg/L). The inhibition has severely affected the whole digester content and it took more than 3 months to recover the process through addition of sodium hydroxide to bring up the alkalinity and pH to neutral (Sulaiman et al., 2001).

In terms of power requirement for digesters mixing system, there is no specific value enforced, however US Environmental Protection Agency (EPA) recommended the power input of 0.20-0.30 HP/1000 cu ft (5.26-7.91 W/m^3) of the digester content (Karim et al., 2005a). In general, the mixing power requirement is very much related to the type of wastewater in terms of total solid contents as confirmed byvarious studies (Karim *et al.*, 2005a; Karim *et al.*, 2005b). For the dilute cow manure of 5% total solids, the influence of mixing was insignificant in terms of biogas productivity for the mixed and unmixed digesters (Karim et al., 2005a). In their study, the biogas productivity and methane yield recorded for the mixed and unmixed digesters were 0.84-0.94 L/L/d and 0.26-0.31 L CH_4/ g volatile solid loaded, respectively.

On the other hand, Sulaiman et al. reported that natural mixing could occur in the digester due to the biogas rising (Sulaiman et al., 2009). They also highlighted that a satisfactory level of methane production could be achieved at 1.0 $m^3/m^3/d$ just by allowing natural mixing without employing any additional mixing devices. Although this value (1.0 $m^3/m^3/d$) was lower than that achieved by the minimal mixing (1.1 – 1.4 $m^3/m^3/d$), but was still higher than the methane production record of only 0.84-0.94 $m^3/m^3/d$ reported by Karim et al. (2005 a-b). The low yield obtained in their study could be attributed to the lower solids content recorded which led to an easier biogas rising due to less resistance available inside the digester. In summary, there is no specific measurement of power requirement for optimum methane production in any particular anaerobic system and it is very much related to the type of wastewater itself whether it is diluted or concentrated. However as a general rule, minimal mixing is the best mode of mixing as confirmed by many studies (Karim et al., 2005a; Karim et al., 2005b; Karaparju et al., 2007; Sulaiman et al., 2009).

2.5 Sludge recycling

As for any anaerobic treatment system designs, it is always desirable to maintain optimum levels of microorganisms inside the digester in order to meet an appropriate ratio of food-to-microorganism (F/M) which results in an efficient bioconversion process. For a complete-mix system Tchobanoglous et al. suggested F/M ratio of 0.2-0.6 IbBOD_5/IbMLVSS/d and slightly lower at 0.2-0.4 IbBOD_5/IbMLVSS/d for a conventional system (Tchobanoglous et al., 1991). In order to maintain high levels of biomass inside the digester, several strategies have been adopted by many researchers. Among those, sludge recycling (Visser et al., 1993; Setiadi et al., 1996; Bae et al., 1998; Faisal & Unno, 2001; Najafpour et al., 2006; Sulaiman et al., 2009; Busu et al., 2010), is probably the simplest strategy to increase the biomass concentration in the digester.

Busu et al. conducted a simple experiment on anaerobic treatment of POME and the sludge was recycled at 6 m^3/d with different feeding strategies. Both feeding strategies (every three and six hours) resulted in a satisfactory COD removal efficiency of higher than 90% but the latter led to a more stable process with the total VFA concentration recorded below 500 mg/l and VFA:Alk ratio of less than 0.3 at the maximum OLR of 6.0 kgCOD/m^3/d applied (Busu et al., 2010). Prior to their study and by using the same digester, Sulaiman et al. proved that by increasing the sludge recycling rate from 6 m^3/d to 12 m^3/d and to the maximum of 18 m^3/d, the anaerobic treatment process could be proceeded to the maximum organic loading rate of 10 kgCOD/m^3/d with biogas and methane productivity of 1.5 m^3 $m^3/m^3/d$ and 0.9 $m^3/m^3/d$, respectively (Sulaiman et al., 2009). Thus based on these two studies on POME, it could be concluded that by increasing the sludge recycling rate during treatment at higher OLRs, the treatment process maintained stable with high COD removal efficiency and satisfactory methane production. Using the same substrate but different digester designs, Setiadi et al., Faisal and Unno and Najafpour et al. revealed that sludge recycling strategy is compulsory to ensure system stability by maintaining pH higher than 6.8 without alkalinity supplementation, eliminating high organic over loading and supplying alkalinity by blending the fresh feed and recycled stream (Setiadi et al., 1996; Faisal & Unno, 2001; Najafpour et al., 2006). Setiadi et al conducted a simple study on the effects of sludge recycling on the baffled system stability and found out that a recycle system of more than 15 times of the fresh feed was required in order to maintain the system's pH higher than 6.8 without alkalinity supplementation and the implementation of the recycle system was an effective means to reduce alkalinity requirements (Setiadi et al., 1996). Najafpour et al. used an upflow anaerobic sludge-fixed film bioreactor and applied a very high recycling ratio of 11.25 of the fresh feed in order to eliminate high organic overloading and to supply alkalinity by blending the fresh feed with the recycle stream characterized by low COD and high alkalinity (Najafpour et al., 2006). As a result, no alkali additional was required to buffer the system. In a different study, Faisal and Unno using modified anaerobic baffled reactor showed that a recycling ratio of 30 was required in order to stabilize the modified anaerobic baffled reactor operation (Faisal & Unno, 2001). On the same concept, but using solid waste substrate, Bae et al. concluded that the continuous addition of active methanogenic population into the anaerobic digester by sludge recycling was effective for rapid and significant methane gas recovery (Bae et al, 1998). Visser et al. also reported that effluent recirculation (of factor 6.7) was necessary in order to improve mixing and to ensure sufficient buffer capacity of extreme pH situation (pH 2-4) (Visser et al., 1993).

2.6 Temperature

It is interesting to note that anaerobic digestion in the natural environments occurred in a wide range of temperatures between 4^oC (lake sediment) to 60^oC (thermophilic digestion process); however, for the industrial practices, the temperature range is limited to 20-55^oC (Fannin, 1987). In the natural environments, the optimum temperature for the growth of methane forming archaea is 5-25°C for psychrophilic, 30-35°C for mesophilic, 50-60°C for thermophilic and >65°C for hyprethermophilic (Tchobanoglous & Burton, 1996). It is generally understood that higher temperature could produce higher rate of reaction and thus promoting higher application of organic loading rate (OLR) without affecting the organic removal efficiency (Desai et al., 1994; Chae et al., 2007; Choorit & Wisarnwan, 2007; Poh & Chong, 2009;). Using palm oil mill effluent as the substrate, Choorit and Wisarnwan, demonstrated that when the digester was operated at thermophilic temperature (55°C), showed higher OLR application than the that of mesophilic (17.01 against 12.25 gCOD /l/d) and the methane productivity was also higher (4.66 against 3.73 l/l/d) (Choorit & Wisarnwan, 2007). A similarly study by Chae et al. indicated that the higher temperature of 35°C led to the highest methane yield as compared to 30°C and 25°C although the methane contents only changed slightly (Chae et al., 2007). Using cheese whey, poultry waste and cattle dung as substrates, Desai et al. showed that when the temperature was increased from 20 to 40 and finally to 60 °C, the gas production and methane percentage in biogas increased as well (Desai et al., 1994). This could be explained by the following reaction rate equation (Tchobanoglous & Burton, 1996). As clearly indicated, as the temperature is raised, the power fraction of (T-20) would be increased as well (as a result of higher enzymatic activity) which could result in higher reaction rate; r_T (see Equation 2).

$$r_T = r_{20}\theta^{(T-20)} \tag{2}$$

Where r_T = reaction rate at T °C, r_{20} = reaction rate at 20 °C, θ = temperature-activity coefficient and T = temperature , °C.

Although the thermophilic anaerobic process could increase the rate of reaction, the yield of methane that could be achieved over the specified organic amount is the same regardless of the mesophilic or thermophilic conditions. That value is 0.25 kg CH_4/kgCOD removed or 0.35 m^3 CH4/kgCOD removed (at standard temperature and pressure i.e 0°C, 1 atm.) which is derived by balancing the following equation (see Equation 3):

$$CH_4 + 2O_2 \longrightarrow CO_2 + 2H_2O \tag{3}$$

On the other hand, although thermophilic condition could result in higher application of organic loading rates and better destruction of pathogens, at the same time it is more sensitive to toxicants and temperature control is more difficult (Gerardi, 2003; Choorit & Wisarnwan, 2007). Furthermore, biomass washout that could lead to volatile fatty acids accumulation and methnogenesis inhibition could also occur if the thermophilic temperature could not be controlled (Poh and Chong, 2009). As a result, in tropical regions, mesophilic temperatures are the preferred choice for anaerobic treatment (Yacob et al., 2005, Sulaiman et al., 2009).

2.7 Alkalinity and pH

As far as the anaerobic digestion process is concerned, it is more appropriate to discuss alkalinity and pH together because these parameters are related to each other and very promising to ensure a suitable environment for successful methanogenesis process. Alkalinity is produced in the wastewaters as results of the hydroxides and carbonates of calcium, magnesium, sodium, potassium or ammonia and may also include borates, silicates and phosphates (Tchobanoglous & Burton, 1991). The alkalinity plays an important pH controlling role in the anaerobic treatment process by buffering the acidity derived from the acidogenesis process (Gerardi, 2003; Fannin, 1987).

Methane producing archaea or methanogens are known to be strongly affected by pH (Poh & Chong, 2009) and could only survive on a very narrow range of pH as listed in Table 3 (Gerardi, 2003). As such, the methanogenic activity will be severely affected once the optimum pH range is not met. Steinhaus et al. studied the optimum growth conditions of *Methanosaeta concilii* using a portable anaerobic microtank (Steinhaus et al., 2007). They reported an optimum pH level of 7.6 revealing that even little variations on both sides of the optimum pH suppressed the growth of the methanogens. Several studies have also reported

Genus	pH range
Methanosphaera	6.8
Methanothermus	6.5
Methanogenium	7.0
Methanolacinia	6.6-7.2
Methanomicrobium	7.0-7.5
Methanosprillium	7.0-7.5
Methanococcoides	6.5-7.5
Methanohalobium	6.5-6.8
Methanolobus	6.5-6.8
Methanothrix	7.1-7.8
Methanosaeta	7.6

Table 3. The optimum pH range for selected methanogens (Gerardi, 2003; Steinhaus et al. 2007)

reactor failure or underperformance simply due to pH reduction caused by accumulation of high volatile fatty acids in the anaerobic treatment system (Visser et al., 1993; Fabián & Gordon, 1999; Poh & Chong, 2009). In a study using synthetic wastewater in the thermophilic temperature, Visser et al. found that at the pH of above 8.0, the methanogenesis was strongly inhibited and the value recorded for acetotrophic methanogenic test was zero (Visser et al., 1993). When investigating the role of pH in anaerobic degradation test, Fabián and Gordon, found out that the acidification led to the low performance of the anaerobic degradation, however the biodegradation was significantly increased once the waste was saturated with water and the pH was adjusted to above 6.5 (Fabián & Gordon, 1999).

There are various types of chemicals that could be introduced into anaerobic digesters for alkalinity supplementation as summarized in Table 4. However on the other hand, over-supplementing these chemical into the system could affect the system in many ways including pH over range, development of vacuum conditions inside the digester, chemical precipitation inside the digester, excess release of ammonia gas, Na^+ and K^+. The last could potentially cause toxicity to microorganisms, lead to foaming problems and increase the oxidation-reduction potential (ORP).

No	Name	Buffering Cation
1	Sodium bicarbonate	Na^+
2	Potassium bicarbonate	K^+
3	Sodium carbonate (soda ash)	Na^+
4	Potassium carbonate	K^+
5	Calcium carbonate (lime)	Ca^{2+}
6	Calcium hydroxide (quick lime)	Ca^{2+}
7	Anhydrous ammonia (gas)	NH^{4+}
8	Sodium nitrate	Na^+

Table 4. Chemicals used for alkalinity supplementation (Fannin, 1987; Gerardi, 2003)

2.8 Ammonia

There are various type of waste/wastewater containing high organic nitrogen such as municipal solid waste, young landfill leachate (Yusof et al., 2009), wastewater from seafood processing factories (Guerrero et al., 1997; Gebauer, 2004), animal waste (Garcia & Angenent, 2009) and waste activated sludge (Appels et al., 2008). Therefore, as a results of degradation of the organic nitrogen fraction, high concentration of total ammonium (ammonium (NH_4^+) plus free ammonia (NH_3) in these waste/wastewater is common (Jokela & Rintala, 2003). Many studies have shown that free ammonia and not ammonium is responsible to inhibit the methanogenic activity during the anaerobic digestion (Sawayama et al., 2004; Sossa et al., 2004; Calli et al., 2005a; Garcia & Angenent, 2009). In a solution, the ammonia exists in a pH dependent equilibrium between ammonium ion (NH_4^+) and unionized ammonia or free ammonia (NH_3) (see Equation 4).

$$Organic\ Nitrogen \rightarrow NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O \qquad (4)$$

The free ammonia concentration is influenced by total ammonium concentration (NH_4^+ + NH_3), pH and temperature as described by equations 5 and 6 (Anthonisen et al., 1976).

$$FA = {}^{17}\!/_{14} \times \frac{TAN \times 10^{pH}}{\left({}^{K_b}\!/_{K_w}\right) + 10^{pH}} \qquad (5)$$

Where, FA (mg/L) is the free ammonia concentration, TAN (mg/L), the total ammonia nitrogen, K_b, the ionization constant of the ammonia equilibrium equation and K_w, the ionization constant of water. The ratio of the ionization constant of the ammonia equilibrium equation and the ionization constant (K_b/K_w) of water is dependant on the temperature (see Equation 6) (Anthonisen et al., 1976).

$${}^{K_b}\!/_{K_w} = e^{\left({}^{6344}\!/_{(273+°C)}\right)} \qquad (6)$$

Therefore, the free ammonia inhibition on methanogens is controlled mainly by total ammonium concentration, pH and temperature. Ammonium ion is an essential nutrient for methanogens growth, however excess concentrations of free ammonia can inhibit these microorganisms. Earlier investigation observed that methanogens inhibition strongly occurred at a wide range of total ammonia nitrogen (TAN) concentrations; 1500-3000 mg/L at pH above 7.4 (Hashimoto, 1986); 3300-4100 mg/L at pH 8.1 and temperature 55°C (Hansen et al., 1998); 6000 mg/L at temperature of 35°C (Calli et al., 2005a) and 5250 mg/L at temperature of 35°C (Garcia & Angenent, 2009). The remarkable differences reported in ammonia inhibitive concentration could be ascribed to variations in the TAN concentrations as well as the operating parameters such as pH and temperature (Sung and Liu, 2003). Besides, the acclimatization of the seed sludge to the elevated concentrations of ammonium also has been reported to contribute to the success of methanogenesis performance (Hashimoto, 1986; Calli et al., 2005a). The adaptation of the methanogens to high concentrations of ammonium was confirmed by detecting resistant methanogens present in the seed sludge with a high concentration of ammonium (Sprott & Patel, 1986). During the adaptation to high ammonium concentrations, it was found that the acetate utilizing methanogens i.e. *Methanosarcina* and *Methanosaeta,* were more sensitive as compared to hydrogen utilizing methanogens (Angelidaki & Ahring, 1993; Sawayama et al., 2004). The *Methanosarcina* sp. was more severely affected by high ammonium concentration than the hydrogen utilizing methanogens (Sawayama et al., 2004). In contrast, a study by Calli et al. an abundant number of *Methanosarcina*-like methanogens at elevated ammonium concentration (6000 mg/l TAN) was observed (Calli et al., 2005b). However, both studies showed that *Methanosaeta*-related species vanished during the adaptation period.

Methanogenesis is more sensitive to ammonia when the pH value is increased. By increasing the pH, greater fraction of the total ammonium will be in the form of free ammonia (NH_3) (eq.1) (Anthonisen et al., 1976) which is believed to inhibit the process and consequently results in low methanogenic activity. The pH of anaerobic digestion reactors treating high organic nitrogen wastes such as poultry leachate and livestock waste were observed high in a range of 7.5-9.0 (Angelidaki & Ahring, 1993; Gangagni et al., 2008). This resulted in 10 times higher free ammonia concentration than the tolerable level and led to the reduction in biogas and methane productions. On the other hand, instability of anaerobic treatment process always results in high VFA concentrations (Strik et al., 2006; Sulaiman et al., 2009). Hence, the low pH encountered will facilitate the reduction of free ammonia concentrations. The relation between free ammonia, VFA and pH is known as inhibited steady state, a condition where the anaerobic process is stable but with low methane yield (Angelidaki & Ahring, 1993).

The anaerobic treatment process has been long known to be influenced by temperature. An increase in temperature will increase the anaerobic degradation rate of the organic substances. It has been shown that the methanogenic activity was higher at higher mesophilic temperature (Masse & Masse, 2001). It was postulated that effects of higher kinetic rate at higher temperature in mesophilic range overwhelmed the free ammonia inhibition (Garcia & Angenent, 2009). However, high ammonia concentrations inhibit methanogenesis more seriously under thermophilic as compared to mesophilic condition (Angelidaki & Ahring, 1993). It was demonstrated that the degradation of swine manure at thermophilic temperature of 55°C was feasible even at high ammonia concentration (6000 mg/l). However, inhibition on methanogenesis was severe which resulted in low methane yield (Hansen et al., 1998). Therefore, although there is a kinetic advantage when operating anaerobic treatment at high temperatures, the thermophilic process is more sensitive to the environmental factors including ammonia toxicity (Sung & Liu, 2003). Higher free ammonia fraction to the TAN has been observed in thermophilic range due to the temperature dependant constant which led to the methanogensis inhibition (El-Mashad et al., 2004; Strik et al., 2006).

2.9 Heavy metals inhibition

Heavy metals are present in various types of waste/wastewater including industrial wastewater, landfill leachate and sludge (Appels et al., 2008; Colussi et al., 2009; Yusof et al., 2009). Although many metals are required in trace amounts to provide sufficient growth to methanogens, the methanogenic activity in anaerobic reactors is strongly affected by excess amounts of heavy metals (Altas, 2009; Colussi et al., 2009). The toxic effects of metals to the biological process is particularly due to the inhibition of enzymes activity as a result of metals binding to the SH group of the enzyme (Nies, 1999). The inhibitory concentrations of four heavy metals on methane-producing granular sludge that caused 50% reduction in cumulative methane production was found to be 7.5 mg/L of Zn, 27 mg/L of Cr, 35 mg/L of Ni and 36 mg/L of Cd with an order of Zn>Cr>Ni≈Cd (Altas, 2009). Whereas a different study revealed that 50% reduction in methane production occurred at 6.4 mg/L of Cu (II), 4.4 mg/L of Cd(II) and 18.0 mg/L of Cr(VI) with an order of Cd(II)>Cu(II)>Cr(VI) in anaerobic digestion of cattail with rumen culture (Yue et al., 2007). The differences reported in the metals inhibitory concentration might be due to the several factors including variation in sludge characteristics, chemical form of heavy metals and microbial resistance to metals (Nies, 1999; Yue et al., 2007; Altas, 2009). Various heavy metals presence in wastewater also showed synergistic effects during anaerobic treatment process. For instance, the presence of chromium in the sludge results in higher toxicity of copper (Colussi et al., 2009).

3. Modeling anaerobic treatment process: New trends

It is well known that the functional and operational state of a bioreactor is subject to wide fluctuations due to process disturbances such as loading rates and pH changes. This has gained considerable concerns within the last years to overcome the alterations and consequently guarantee the safe operation of anaerobic digesters. The attention toward such issues gets more crucial when high rate bioreactors are utilized. On the other hand, an exact picture of what happens in bioreactors has been so much sophisticated and as a result, massive simplifications have been assumed to predict the necessary outputs of the process (Lin, 1991). Mathematical modeling of wastewater treatment processes plays an outstanding role as a tool capable of providing diagnostics that will give support to the plant operation and the decision-making process.

3.1 Artificial neural networks

Many analytical models, mostly kinetic models, have been developed to describe the anaerobic treatment in bioreactors, however, they are not routinely used for control and on-line applications. The reason lies in their complexity and insolvable parameters. Furthermore, the kinetic models are highly affected by the environmental conditions rendering them to be too generalized for other substrates or environments (Tay & Zhang, 2000).

Artificial neural networks (ANN), as powerful modeling methods have found a great deal of interest among the researchers in the last two decades (Feng et al., 2007; Zeng et al., 2003). They can solve a wide range of problems and differential equations, particularly when the conventional approaches fail (Ghobadian et al., 2009). The ability of ANNs in the modeling is traced back to their training mechanism which is inspired by biological neurons (Fig 2 a). Fig 2b presents a three-layer network. The data processing can extend over multiple layers and the final error criteria such as mean squared error (MSE) are calculated at the output layer which measures the accuracy of modeling (Hertz et al., 1991).

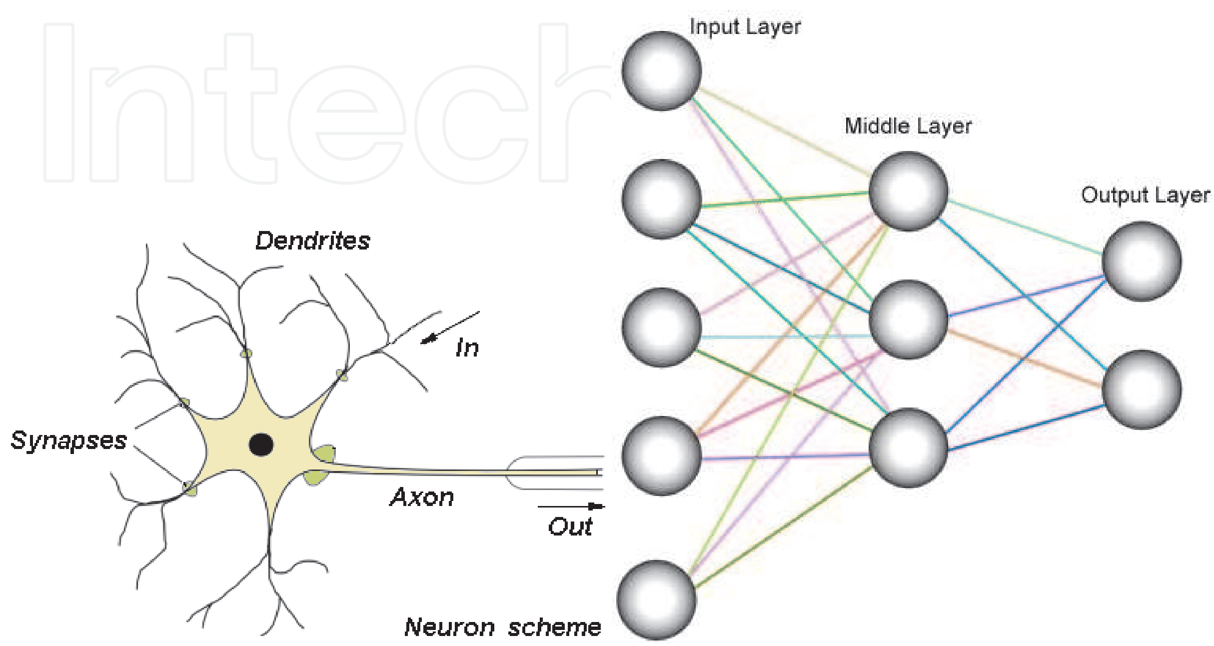


Fig. 2. a) a schematic picture of biological neurons, b) representation of biological neurons as a feed-forward three-layer network.

A well trained network learns from the pre-seen experimental dataset (training data) and generalizes this learning beyond to the unseen data which is called 'prediction' (Haykin, 1994). Furthermore, ANNs are able to model non-linear behaviors and complex processes. This is highly important in anaerobic treatment and bio-processes due to the special hydrodynamics and non-linear nature of the anaerobic digestion (Holubar et al., 2002). Based on the mechanism of human nervous systems, the ANN models can be classified into three major groups; 1) feedforward network, 2) recurrent network and 3) unsupervised network (Fig 3). The most usual model is feedforward network which has frequently been used in anaerobic treatment studies (Zhu et al., 1998; Gontarski et al., 2000; Strik et al., 2005; Ozkaya et al., 2007; Parthiban et al., 2007). Although, recurrent and unsupervised networks are powerful models developed to map the variables of anaerobic processes non-linearly, a few studies have applied them in on-line or control applications (Kecman, 2001). This could be ascribed to the simplicity, accuracy and swiftness that feedforward networks offer (Kalogirou, 2000, Kiani Deh Kiani et al., 2009).

ANN together with other intelligent methodologies could be a promising alternative to the conventional techniques. Of the premier studies of this field, was the investigation carried out by Zhu et al., on modeling the wastewater process by time-delay neural networks in order to predict the quality attributes of the process (Zhu et al., 1998). A few years later, Gontarski et al. also proved the successful application of neural networks in the simulation of industrial anaerobic treatment plant in Brazil. They showed that the liquid flow rate and pH of the inlet stream were the major variables in controlling the plant and the neural network presented desirable results in minimizing the plant fluctuations (Gontarski et al., 2000). In a different study, a hybrid technique providing principal component analysis (PCA) together with neural networks was used for optimal control of a wastewater treatment process (Choi & Park, 2001). The application of PCA in that case emerged as a novel idea at the time, since the input dataset could be reduced in order to solve the overfitting problem of the model. Zeng et al. employed back-propagation multilayer perceptrons (MLP) networks to model the nonlinear relationships between the removal rates of pollutants and the chemical dosages. Using this technique, the system could be adapted and operated in a variety of conditions showing a more flexible performance (Zeng et al., 2003).

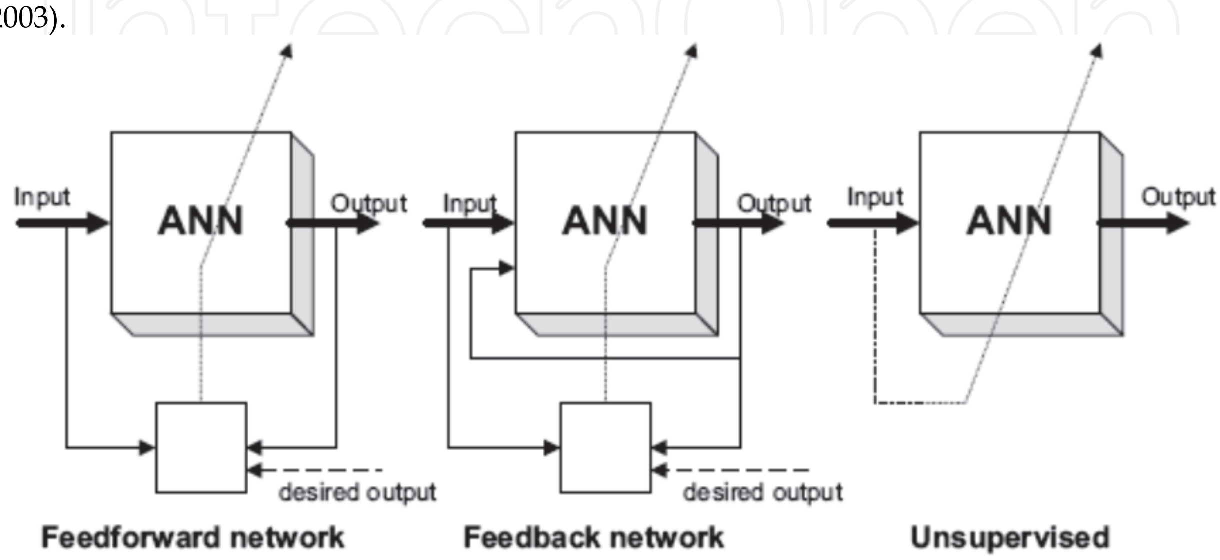


Fig. 3. Three general models of neural networks used in engineering analyses (Timothy Hong et al., 2003)

A few studies have reported the utilization of unsupervised learning algorithms. Hong et al. applied Kohonen Self-Organizing Feature Maps (KSOFM) neural networks to analyze the process data obtained from a municipal wastewater treatment plant (WTP) (Timothy Hong et al., 2003). KSOFM differ from feedforward networks in a way that they provide a clustering methodology which leads to data reduction (Kasabov, 1998) and also project the data nonlinearity onto a lower-dimensional display. The latter creates an abstraction of various features of input signals in the absence of any supervisor signals (Kasabov, 1998). As the authors reported, the KSOFM was computationally efficient, accurate, and reliable for the analysis of WTP and since the analysis and diagnosis of WTPs is a difficult task, the developed technique yields a great deal of significance (Timothy Hong et al., 2003). There are also some other studies which have utilized unsupervised networks for modeling the wastewater treatment process (Hong & Bhamidimarri, 2003; Garcia and Gonzalez, 2004; Cinar, 2005).

Tay and Zhang developed a fast predicting neural fuzzy model to predict the response of high-rate anaerobic systems to different system disturbances 1 h in advance (Tay & Zhang, 2000). Three laboratory scale systems including an anaerobic fluidized bed reactor (AFB), an anaerobic filter (AF), and an up-flow anaerobic sludge blanket (UASB) reactor were utilized. The reactors underwent two disturbing shocks, organic loading rate and hydraulic loading rate. The adaptive network based fuzzy system (ANFIS) used a database of system performance and implemented these data to predict the response of the anaerobic wastewater treatment system in the presence of OLR, hydraulic loading rate (HLR) and alkalinity loading shocks. The adaptability of the neural fuzzy modeling used was proven to be acceptable in different operation conditions and therefore, it was suggested to be of high potential in real time control (Tay & Zhang, 2000).

Moreover, advanced controlling of anaerobic digestion has been also achieved using hierarchical neural networks (Holubar et al., 2002). Holubar et al. developed several configurations of feedforward back-propagation neural networks to predict gas composition, methane production rate, volatile fatty acid concentration, pH, redox potential, volatile suspended solids and chemical oxygen demand of feed and effluent of a CSTR under pulse like disturbances of OLRs. The correlation coefficient between the measured data and calculated values were found to be bigger than 0.8 for all the predicted variables which was a promising result by which the controlling of CSTR could be accomplished.

On the other hand, hydrogen sulfide (H_2S) and ammonia (NH_3) as the main gaseous trace compounds in anaerobic digestion process were predicted in a CSTR digester by using ANN (Strik et al., 2005). The obtained coefficients of determination (R^2) were 0.91 and 0.83 for hydrogen sulfide and ammonia, respectively. A similar research was conducted on an AFB reactor for starch wastewater using MLP and the effects of OLR, hydraulic retention time (HRT) and efficiency of the reactor on its steady-state performance were modeled using ANN (Parthiban et al., 2007). The MSE of the network performance was found the desirable value of only 0.0146. The findings of this research were of a great value since effluent COD and pH, alkalinity, VFA and biogas production were predicted by two input parameters: influent pH and OLR.

Neural networks have shown a more extensive application predicting methane fraction in biogas produced by field-scale land-fill bioreactors (Ozkaya et al., 2007). The methane production was modeled based on inputs such as pH, alkalinity, COD, sulfate, conductivity, chloride and substrate temperature. As a result, predicting hourly methane production, control achievement, optimization of energy conversion and construction time could be achieved. Pai et al. employed Grey Model ANN (GM-ANN) to predict suspended solids (SS) and COD of hospital wastewater treatment reactor effluents (Pai et al., 2007). Results showed that GM-ANN could predict the hospital wastewater variations with the same accuracy as ANN did. Besides, while ANN needed a large quantity of data, GM-ANN could handle smaller quantities.

In another study, a real-time monitoring of wastewater treatment process was achieved with the aid of multivariate statistical methods and ANN (Luccarini et al., 2010). In that study, Luccarini et al. installed some probes for the acquisition of signals such as pH, ORP and dissolved oxygen in a SBR and manipulated these data in an ANN model. The research aimed to verify the treatment process based on the continuous signals obtained on-line from the reactor (Luccarini et al., 2010). The signals from the plant were transmitted by telecommunication facilities and the data were reduced using PCA method and then analyzed. Therefore, by remote monitoring of a small-scale reactor ANN could approximate the performance criteria of the plant on-line. This novel work opened a new window for the remote controlling of reactors and benefiting ANN potentials to model such facilities.

3.2 Genetic algorithms

Genetic algorithms (Ga) fall in a class of stochastic search strategies modeled after evolutionary mechanisms based upon evolutionary principles of natural selection, mutation, and survival of the fittest (Sivanandam and Deepa, 2008). They have become a very popular strategy to optimize non-linear systems with a large number of variables. Gas are very different from most optimization methodologies comprising well-defined algorithms. The GA approach is to generate a large number of potential solutions and “evolve” a solution to the problem. One of the big keys to a successful genetic algorithm is in the development of a good "fitness function". The fitness function is how each potential solution is evaluated by the algorithm, and is in essence, how the problem to be solved by the algorithm is defined (Melanie, 1999). Optimization of wastewater treatment variables using GA models was reported by Cho et al. (2004). In their study, GA was integrated with a mathematical management model to reduce the treatment costs at a Korean plant and the application of GA was promising as concluded by the authors (Cho et al., 2004).

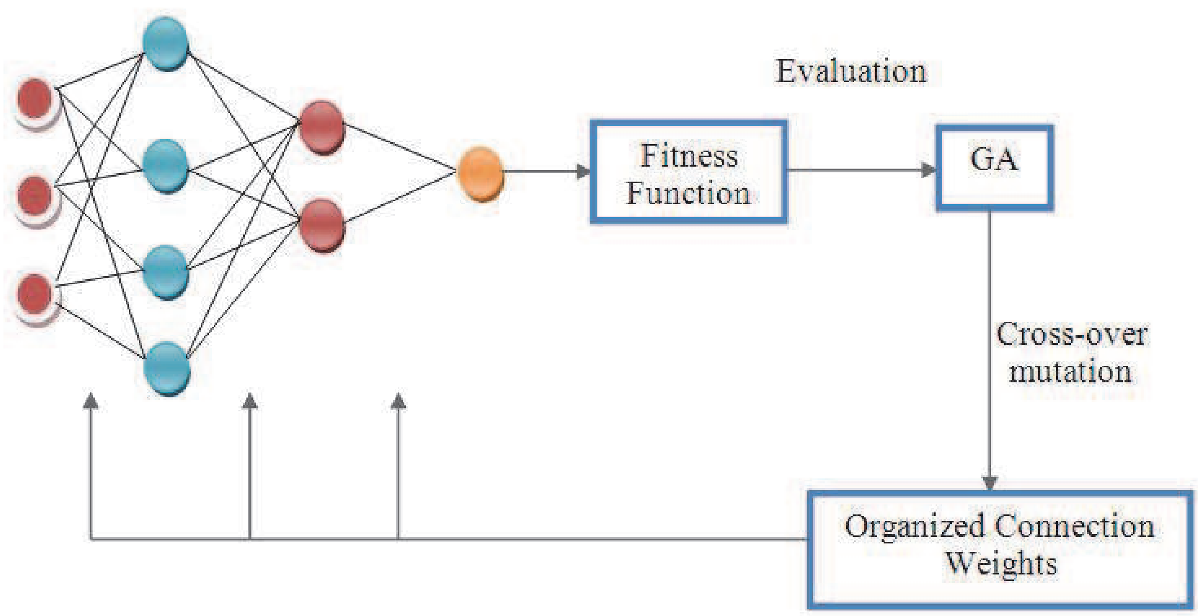


Fig. 4. Overview of the GA-ANN model

There have been several hybrid systems developed that combined neural networks with Gas in various ways (Haupt & Haupt, 2004). These generally fall into three categories: (1) using a GA to determine the structure of a neural network (Kecman, 2001), (2) using a GA to calculate the weights of a neural network as depicted in Fig 4, and (3) using a GA to both determine the structure and the weights of a neural network (Sivanandam & Deepa, 2008). Yang Mu and Yu developed a hybrid model for simulation of biological hydrogen production in a UASB reactor was reported (Yang Mu & Yu, 2007). OLR, HRT, and influent bicarbonate alkalinity were fed to a model of ANN combined with a GA. H_2 concentration, H_2 production rate, H_2 yield, effluent total organic carbon, and effluent aqueous products including acetate, propionate, butyrate, valerate and caporate were determined as outputs of the model. Simulations showed that the model could describe the daily variations of the studies UASB reactor by predicting the steady-state performance of reactor regarding various HRTs and substrate concentrations. Hybrid models have also been studied on the biodegradation process of phenol in a FBR. The authors suggested that the feedforward ANNs trained by a real-coded GA acted desirably for the simulation of biodegradation process in the FBR (Venu Vinod et al., 2009). In a different study, Iqbal and Guira developed an optimization procedure for an activated sludge reactor. They built a multi-objective optimization (MOO) to take into consideration the maximized influent flow rate of wastewater and minimized exit effluent biological oxygen demand (BOD) (Iqbal & Guriaa, 2009). The correct utilization of this method would be a necessity in the treatment process modeling and optimization as the non-linear and complex behavior dominating the bioreactor performance requires.

3.3 Fuzzy systems

Fuzzy logic, a novel technique introduced in 1965 by Lotfi Zadeh, is a mathematical tool for dealing with uncertainty and relative importance of precision. It is a convenient way to map an input space to an appropriate output space (Kasabov, 1998). The basis for fuzzy logic is similar to that for human communication or natural language. This statement underpins the whole concept of the technique. The theory of fuzzy logic is based upon the notion of relative graded membership and so are the functions of cognitive processes. The utility of fuzzy sets lies in their ability to model uncertain or ambiguous data so often encountered in real life, esp. biological issues (Sivanandam et al., 2007). At present, numerous applications of fuzzy logic in control and automation exist and many devices, plants and industrial facilities are instrumented with fuzzy control systems (Kandel & Langholz, 1993). However, designing fuzzy logic controls would require a series of prerequisites, including the determination of the input and output variables, the parameters of membership functions, and the fuzzy control rules (Chen et al., 2003). In spite of the extensive application in several areas of industry and research, fuzzy logic control has not been so popular in anaerobic digestion processes. Moreover, the need for a proper tool for screening out the essential control rules based on the experimental knowledge about the plant operation seems beyond the question. In order to overcome this challenge, Chen et al. conducted a three-stage study using fuzzy-neural hybrid controller for industrial wastewater treatment (Chen et al., 2003). The first stage was identifying the state function of wastewater treatment system followed by searching for multi-objective control strategies, and finally, tuning fuzzy control rule base. The results of the simulations proved that the hybrid fuzzy control approach effectively achieved the required real-time control objectives and was shown as an efficient and cost-effective tool to deal with the unexpected uncertainties in the wastewater treatment process (Chen et al., 2003). The authors also stated that their control architecture could be generalized to other physical, chemical and biological waste treatment systems.

In a different investigation, fuzzy-neural control system was utilized to real-time control and supervise the submerged biofilm wastewater treatment reactor (Mingzhi et al., 2009a). The research aimed to maximize cost efficiency of the treatment operation and address the problem of controlling air flow rate. The results obtained indicated that using fuzzy logic combined with ANN models led to better outputs and less MSEs compared to pure ANN. As a general conclusion, the authors reported that the fuzzy-neural control system used performed desirably when dealing with unexpected uncertainties in the small-scale bioreactor (Mingzhi et al., 2009a).

Mingzhi et al. developed a fuzzy neural network to model the nonlinear relationships between the removal rate of pollutants and their chemical dosages in a paper mill wastewater treatment plant. The objective of their research was to adapt the system to a variety of operating conditions and also to achieve a more flexible performance. The developed model reached a reasonable prediction of the COD and BOD in a high efficient reactor (Mingzhi et al., 2009b). A similar study was conducted by Pai et al. which employed fuzzy systems in combination with neural networks to predict SS and COD in the effluents of a hospital wastewater treatment. Regarding the maximum coefficient of correlation (R) and the minimum mean absolute percentage errors (MAPE) of the predictions, the developed model showed a satisfactory performance in comparison with the pure ANN models. Therefore, the model developed could be recommended in order to optimize design considerations of the treatment process (Pai et al., 2009).

Model	Application Domain	Advantages	Drawbacks	Reported applications in wastewater treatment
ANN	Modeling-regression analysis, classification, clustering,	Models non-linear data, Adaptive nature, robustness, parallel nature (continues to work when an element fails)	Needs training, high processing time for large networks, subject to over-fitting and under-fitting	modeling the wastewater process by time-delay neural networks (Zhu et al., 1998), optimal control of a wastewater treatment process integrated with PCA (Choi and Park, 2001), Kohonen Self-Organizing Feature Maps (KSOFM) to analyze the process data of municipal wastewater treatment plant (Timothy Hong et al., 2003), Unsupervised networks for modeling the wastewater treatment process (Garcia and Gonzalez, 2004; Hong and Bhamidimarri, 2003; Cinar, 2005), Grey Model ANN (GM-ANN) to predict suspended solids (SS) and COD of hospital wastewater treatment reactor effluents (Pai, 2007), on-line monitoring of a reactor (Luccarini, 2010)
FUZZY	Control systems, automation, modeling, integration with ANN for classification	Not constrained to the crisp logic, benefits from linguistic nature	Hard to implement in machines and practical systems	Fuzzy-neural hybrid controller for industrial wastewater treatment (Chen, 2003), control and supervise the submerged biofilm wastewater treatment reactor (Mingzhi et al., 2009), modeling the nonlinear relationships between the removal rate of pollutants and their chemical dosages in a paper mill wastewater treatment plant (Mingzhi et al., 2009).
GA	Optimization, control engineering, integration with ANN	Mimics the process of natural evolution, desirable for problem domains that have a complex fitness landscape	requires expensive fitness function evaluations for complex high dimensional, multimodal problems, subject to converge toward local optima,	GA-ANN for simulation of biological hydrogen production in a UASB reactor (Yang Mu and Yu, 2007), GA-ANN for biodegradation process of phenol in a fluidized bed reactor (FBR). (Venu Vinod et al., 2009), multi-objective optimization (MOO) for optimization procedure for an activated sludge reactor (Iqbal and Guriaa, 2009)

Table 5. Summary of introduced models with examples and aspects to be considered

Table 5 summarizes the aforementioned models together with the advantages and drawbacks which might be considered for selection in applied projects and utilization in industrial scales. As observed the most extensively used model is ANN. The reason is already discussed in this chapter. However, the application domain of Fuzzy logic and Genetic algorithms provide a high potential for integration of these models with ANN. Fields such as optimization and classification can be highly enhanced when integrated models are applied.

4. Conclusion

This chapter reviewed and discussed various influential parameters on biomethane generation during anaerobic treatment of wastewaters i.e. granulation, OLR, LCFA, mixing, sludge recycling, temperature, alkalinity and pH, ammonia and heavy metals inhibitions. By fully understanding these factors, anaerobic plant operators could enhance the process performance for improved biomethane generation and COD removal. Besides, the successful operation of anaerobic digesters also involves efficient process control component as the anaerobic treatment process relies on the complex biochemical reactions involved. This has led to the development and consistent improving of the anaerobic mathematical modeling techniques to further enhance the operation. In this chapter, the applications of modern modeling techniques such as ANN, GA and Fuzzy system as capable tools for optimizing the plant operation and assisting in the decision-making process were also discussed in detail.

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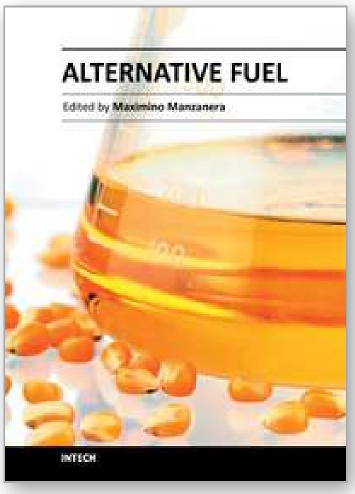

Alternative Fuel

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Renewable energy sources such as biodiesel, bioethanol, biomethane, biomass from wastes or hydrogen are subject of great interest in the current energy scene. These fuels contribute to the reduction of prices and dependence on fossil fuels. In addition, energy sources such as these could partially replace the use of what is considered as the major factor responsible for global warming and the main source of local environmental pollution. For these reasons they are known as alternative fuels. There is an urgent need to find and optimise the use of alternative fuels to provide a net energy gain, to be economically competitive and to be producible in large quantities without compromising food resources.

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